Summary of HESI in vitro ADME Workshop and in vitro Test Types

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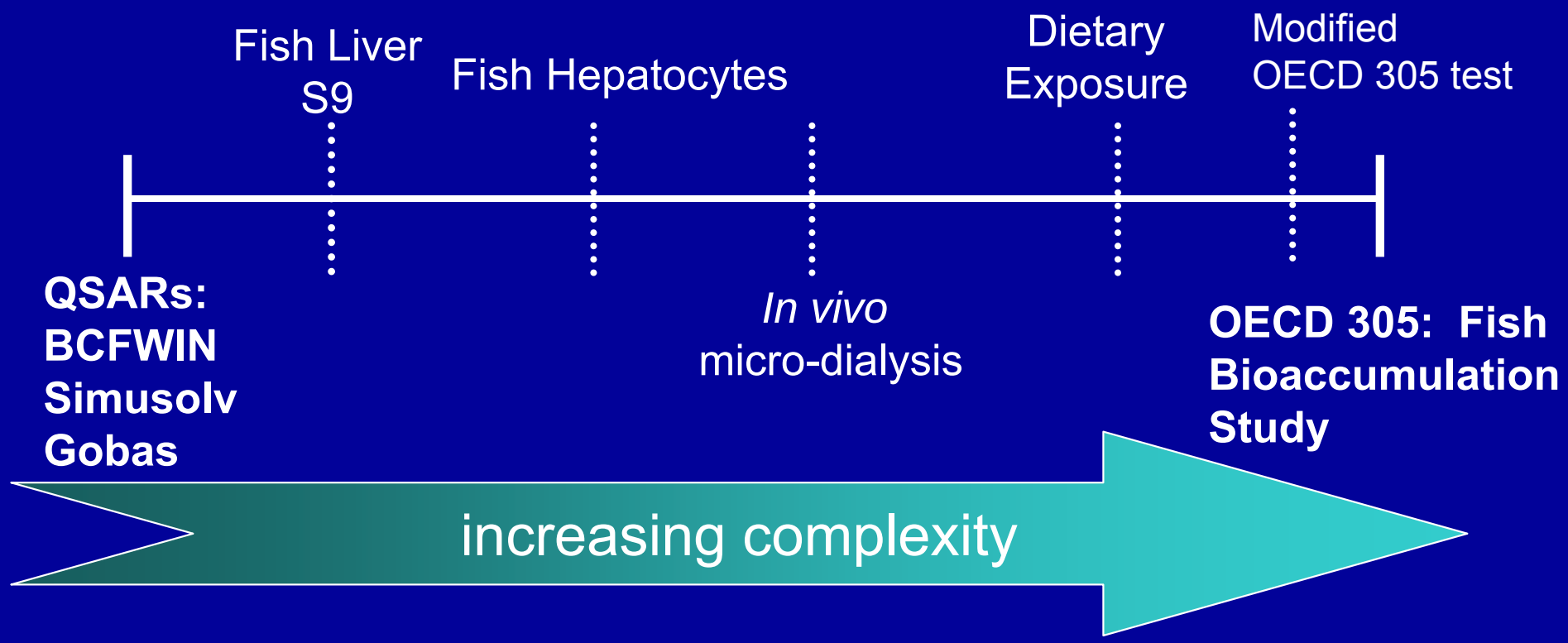
Presentation Overview

- History and need behind the ADME workshop
- Workshop Overview and organization
- Workshop goals and objectives
- Workshop findings and recommendations
  - Research Strategy to promote and develop in vitro tests (Birgit Hoeger)
    - Critical areas of research
    - In vitro tests specifics
  - How might in vitro data be incorporated into B assessments: A Decision Tree
- Concluding remarks

ILSI-HESI Emerging Issues-Bioaccumulation Working Group

- Academics, industry and government/regulatory agencies
  - Representing North American and European Perspectives
- Organizational workshop held April 2005
- Five Issues Identified
  - **integrate in vivo and in vitro ADME data into 'B'**
  - communicate the state of the needs of 'B' science broadly
  - improve 'B' models and databases
  - improve laboratory to field extrapolations
  - extrapolate across species

Current State of BCF Determination



A Tiered Approach to Bioaccumulation Assessment

a) phys-chem analyses; literature search,
b) computer models to estimate ADME & BCF

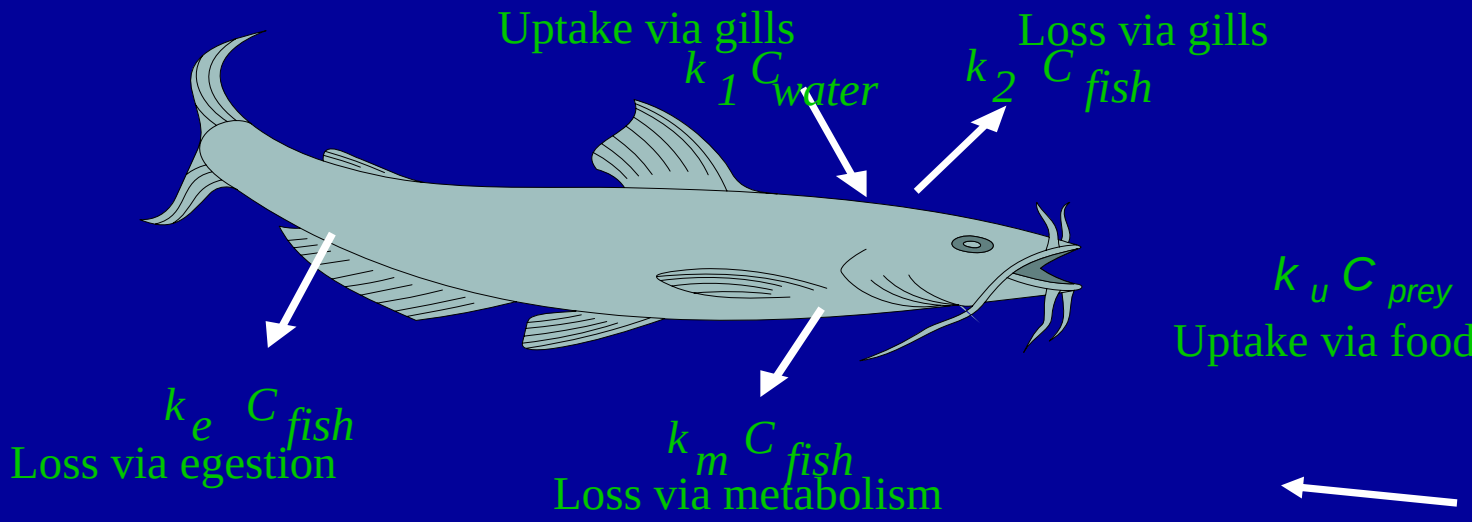
Tier II

***a) In vitro* methods to evaluate absorption, metabolism, or BCF in cellular or subcellular fractions**

b) Use data generated *in vitro* for refined modeling scenarios

In vivo methods to measure bioaccumulation

A&M parameters in ‘B’ model?

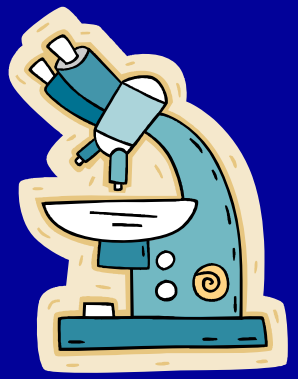


BAF / ϕ = (k_1 + k_U β τ L_D K_{OW}) / (k_2 + k_E + k_M + k_G)

Using defaults: BAF = k_1 + k_U (0.7) K_{OW} / k_2 + k_E

- Pharmaceutical in silico models can estimate high vs. low k_1 and k_M
- *In vitro* tests in combination with extrapolation can deliver k_1, k_M, k_U, k_2, k_E.

ADME Workshop: Organization



- STATE of the SCIENCE
  - Where are we now?
- Breakout session I: – Where do we want to go?
- What are the data gaps and how do we address them?
- Breakout session II: What should be the research strategy and how might in vitro data be incorporated into B assessments?

Participating Organizations

Private

- ADMET Technologies, Inc.
- 3M
- Cellz Direct
- Dow Corning Corporation
- Dupont
- EcoToxicology
- L'Oreal, ***France***
- Nova Chemicals
- The Dow Chemical Company
- The Proctor and Gamble Corporation
- Vizon SciTec Inc

Academic

- Louisana State University
- Ohio State University
- Simon Fraser University, ***Canada***
- The University of Texas at Austin
- University de Montreal, ***Canada***
- University of Bern, ***Switerland***
- University of Florida

Government

- ECVAM, ***Italy***
- NOAA/Great Lakes Environmental Research Lab
- Battelle PNNL-Marine Research Operations
- UA EPA/ Environmental Effects Laboratory
- Environment Canada, ***Canada***

In vitro ADME Workshop Goals

- Determine the state-of-the-science for use of *in vitro* data in bioaccumulation assessments for fish including:
  - use of *in vitro* and *in vivo* ADME information in current bioaccumulation models for fish;
  - application of *in vitro-in vivo* metabolism extrapolation methods
  - identification of *in vitro* experimental methods
  - development of new *in vitro* experimental models for the estimation of biotransformation and bioavailability in fish, extrapolation of ADME data

Workshop Objectives

- Identification of knowledge and research gaps that preclude an expanded use of *in vitro* data in bioaccumulation assessments
- Standardization of *in vitro* test methods to achieve greater comparability among studies and uniform application of the data, especially in the regulatory arena
- Prioritization of future research and training needs,
- Production of a workshop report which makes specific recommendations concerning the collection of *in vitro* data and its use in bioaccumulation assessments for fish

ADME Workshop: **State of the Science**

PRESENTATIONS

- **Bioaccumulation overview** -Annie Weisbrod – Proctor and Gamble
- **In vitro techniques in drug discovery** – Jasminder Sahi, Cellz Direct
- **In vitro applications in fish – techniques and limitations** Helmut Segner (University of Bern, Switerland) and Margaret James (University of Florida)
- **The Canadian Perspective assessing B** – John Nichols (US EPA) and Mark Bonnell (Environment Canada)
- **Modeling and extrapolation of in vitro data in fish** – Irv Schultz, Batelle Labs, PNNL)

Highlights from Presentations

- *in vitro* techniques have already been developed to describe ADM and E and are widely accepted in the field of toxicology
- Many have been or can be adapted to fish
- Scale up from *in vitro* metabolism to the whole organism is possible using standardized calculations
- Standardization of *in vitro* protocols is key
- “B” categorization was hampered by lack of experimental data during CSDSL Experience
- Current modelling scenarios are sufficient as long as metabolism is included in estimation of “B”

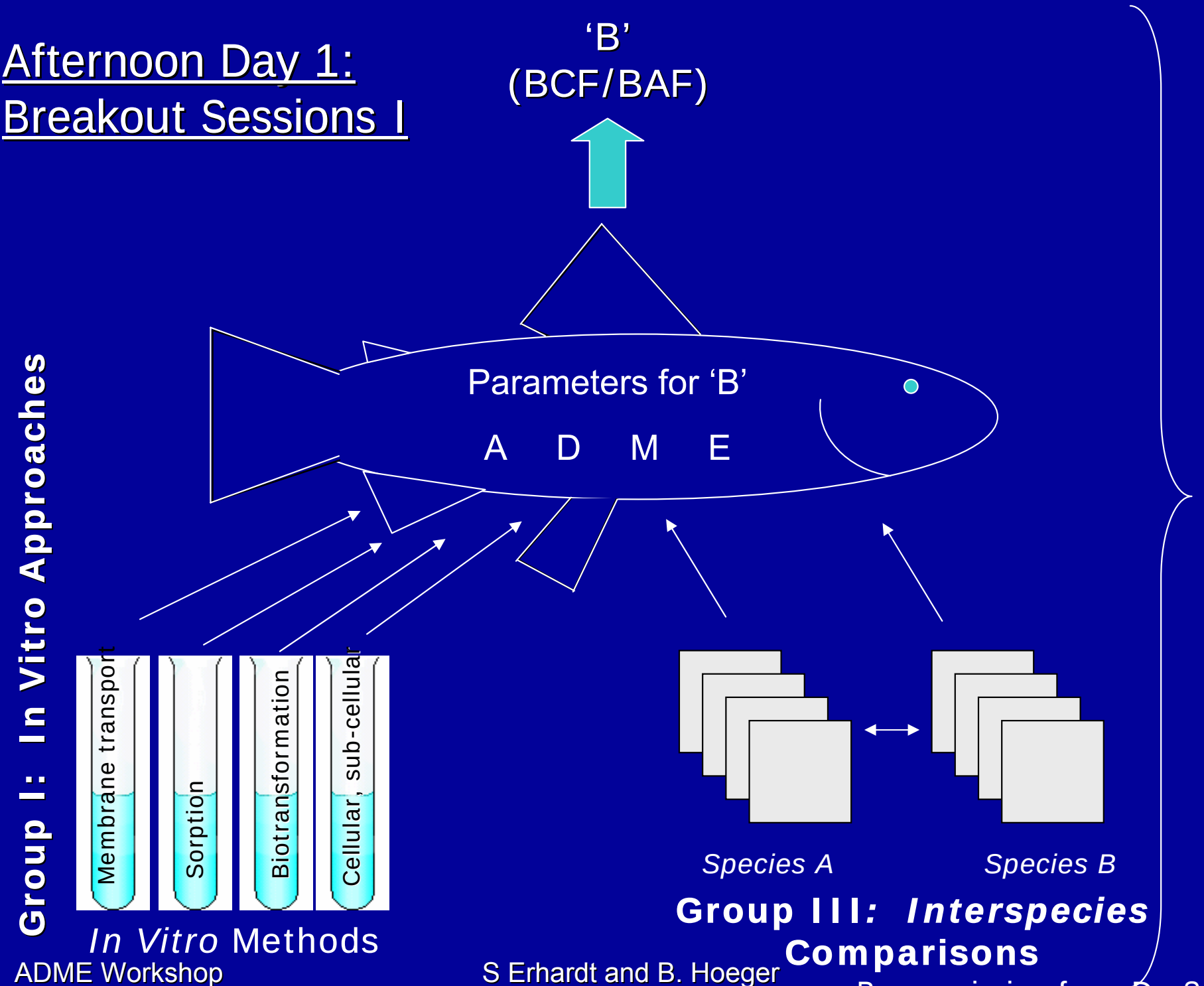
Extrapolation to the Whole Organism with S9 *in vitro* data

Chemical (Log Kow)	Measured BCF (OECD 305)	Modeled BCF (Gobas)	Measured k_M (day^{-1}) (OECD 305)	Modeled from S9 k_M (day^{-1})*
Chlorpyrifos (4.96)	1400	1380	0.25	0.4
Fluroxypyr-MHE (1.17)	25	2	3.2	9.15
Haloxyfop-ME (3.38)	13	34	11	5.97
Zoxamide (3.90)	400	107	1.7-1.9	2.12

***Assumptions:**

- "Modeled k_M" calculated using measured trout S9 metabolism
- Volume of distribution remains constant at 3.5
- All protein in solution is "active"
- All chemical enters target tissue; no reduced permeation.

Afternoon Day 1: Breakout Sessions I



By permission from Dr. S. Dyer

Group I: In Vitro Tests – key findings

- **Determine the influence of bioavailability on uptake and distribution**
  - poorly understood especially in fish
  - Use abiotic tests to determine bioavailability as tier I
    - SPME, PAMPA or EVA
- **Adapt Lipinski's rule of five for fish (C. A. Lipinski, et al., 1997)**
  - defines uptake utilizing physico/chemical means in combination with in vitro data similar to pharmaceutical approach
- **Develop a basic screen of Kow can be used to determine cut off…**

  THEN move to biological system
- **Focus on liver as primary organ of interest for metabolic clearance**
  - consistent with current approaches for pharmaceticals
- **Development of standardized tests critical**
  - little consistency on how in vitro tests are conducted
- **Animal reduction but not animal elimination possible at this time**

Group II: Application of in vitro data – key findings

- **existing mathematical models for fish do an adequate job of predicting B potential**
  - limted to organic compounds, provided that they are poorly metabolized.
  - Recent models have become increasingly “physiological”,.
- **The greatest limitation of existing B models is a lack of chemical metabolism rates.**
  - likely metabolic products from parent chemical structure can be modeled; however no rate information at this time.
- **Critical Gaps:**
  - standardized approach, and validation of *in vitro* methods for predicting metabolism
  - strategic selection of common test compounds

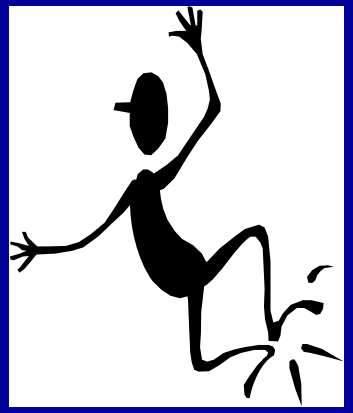
Group III: Interspecies Comparisons – key findings

- **extrapolations of physiological and biochemical information among fish species are feasible, while fish-to-mammal extrapolations carry significant uncertainty**.
  - Little known about differences in metabolism or bioavailability between fish and mammals
  - Need to compare enzymatic and biological membrane properties among these phyla before extrapolation employed
- **A "crop grouping" approach was endorsed as a potential means for performing fish-to-fish extrapolations.**
  - utilize 3-5 fish species (e.g., warm, cool, and cold water species).
  - Over time, a database to relate *in vitro* to *in vivo* outcomes could be developed for these few species and extrapolated to a more diverse set of fish

What are the critical research and data gaps?

- **The effect of bioavailability** on uptake and bioaccumulation
- **Adaptation of physico/chemical properties** to estimate uptake as a first level of screening in addition to QSAR estimates
- **Limited data comparing *in vitro* with *in vivo* BCFs**
- **Standardized** protocols and cross lab validation
- **Cross species comparisons** between fish species
- **Guidance** on use of *in vitro* data once it is generated

Afternoon Day 2: Breakout Session II



- Three groups
  - Bioaccumulation Overview
  - Research Strategy
  - Decision Tree
- Optional for participants to contribute
- ~2/3 of participants stayed and contributed to discussions and final documents
- Level of Enthusiasm and participation from the group **fantastic!**

Breakout Session II: Application of *in vitro* data

***Session Charge*:**

Design a research strategy that will lead to determination of how and when diverse in vitro methods may be used

- Review current use of *in vitro* data in bioaccumulation models for fish
- Discuss potential new applications of *in vitro* information
- Identify knowledge gaps that preclude expanded use of this data
- Suggest experimental or other approaches to fill these gaps

***Expected outcome*:**

Rational and prioritised list of research needed to advance the use of *in vitro* data in bioaccumulation models for fish

Breakout Session II: Application of *in vitro* data

Selection of chemicals

- Suitability of methods may depend on types of chemicals assessed
- Factors that can affect the choice of chemicals: solubility, sorption, volatility, potential for biotransformation, metabolic pathways, availability of analytical methods and existence of reliable 'B' data (with species noted)

Several analysis comparing Kow-only based 'B' to measured 'B' values available → provide much of the needed data to select chemicals for *in vitro* method evaluation

Breakout Session II: Application of *in vitro* data

Factors that can be used for chemical selection for the demonstration project

Chemical	'B' (Kow-only)*	Measured 'B'**	Predicted *Kmet****	Species	Predicted Metabolic Pathway(s)*****	Analytical Method Available******
A	High	< Kow-only	High	From measured 'B' data	Phase I and II	
B (Real 'B')	High	~ Kow-only	Low		Phase I	
C	Low	< Kow-only	High		Phase I and II	
D	Low	~ Kow-only	Low		None	

* 'B' (Kow-only) = predicted 'B' based on Kow with no metabolism in the estimate
** 'B' is a measured value from reliable tests
*** 'Kmet', rate of whole-fish metabolism (from Gobas & Arnot), either quantitatively predicted or qualitatively estimated from comparing predicted vs. measured 'B'.
****Metabolic pathways for mammals are available via best professional judgment and models, such as Meteor. However for fish models, are not available, hence potential pathways will be based on best professional judgment

Breakout Session II: Application of *in vitro* data

Systems that describe exposure and dose

Systems that can provide measures of sorption or bioavailability are critical!

Abiotic Systems

- EVA (ethylene vinyl acetate film)
- SPME (solid phase micro-extraction)

→ **determine amount of test material sorbed and free in any media**

→ **should be used in sub-cellular and cellular media, water (for fish tests), fish blood**

Biotic Systems

- Caco-2 (discussed)
- Fish intestinal preparations

(favoured, as relates directly to fish physiological processes; "fish are not 'wet mammals'")

in Vitro 'M' Assays

Cellular preps

Culture:
Carp at 24°C
Trout at 17°C

liver

Hepatocytes (30°C)
Test in suspension (<24 hr)

Homogenisation 600g, 10 min

SUPERNATANT

Ultracentrifugation 10 000g, 20 min

SUPERNATANT

Subcellular preparations

Homogenate suspension
Both cytosolic as well as microsomal fraction of cells and thus all the Phase I and Phase II enzymes.

S9 fractions (-80°C)
Phase I and II enzymes
larger debris removed including protease inhibiting enzymes

Ultracentrifugation 10 000g, 20 min
Homogenisation

Microsomes
Vesicles containing the CYP450 system enclosed by a mainly complete membrane system.

Incubations 60 min at 25°C

Breakout Session II: Application of *in vitro* data

Sub-cellular Systems

- S9 (potential to assess both Phase I and II reactions)
- Microsomes (biased to Phase I biotransformation)
- Liver homogenates (was not considered worthy of further consideration)

→ Screening tools to assess potential for biotransformation in higher biological systems (cellular, tissue and whole fish)

→ biotransformation rates can be measured, as metabolite generation and/or loss of parent material

→ results should be communicated as positive/negative (yes/no) or binned (high/medium/low) potentials

Ease of development, cryo-preservation possible →
potential for rapid transfer between labs
But at the moment no standardised methods for fish!!!

Breakout Session II: Application of *in vitro* data

Cellular Systems

'B' materials likely enter the fish via the intestine

→ need to assess the importance of intestinal uptake and metabolism

- Fish intestinal preparations (require method development)
- Fish hepatocytes

Hepatocytes include membrane transport (active, passive), Phase I and II reactions

Use in plated or suspended form possible

Limitations:

Current use is limited to few lab species (Common carp and Rainbow trout)

Need for fresh culture

Tissue yield

Cryo-preserved fish hepatocytes? (already commercially available for mammals and humans)

Breakout Session II: Application of *in vitro* data

In situ isolated liver perfusion

- Provide greatest integrative measure of uptake, distribution and biotransformation of chemical in the liver
- Have only been developed for catfish and Rainbow trout
  (species with encapsulated livers and clearly defined hepatic and portal blood vessels)
- Exposure should be based on free and total fractions as dosed via blood

Breakout Session II: Application of *in vitro* data

Modelling

Estimates of 'B' can be made via simple box models as well as more complex PBTK-type models

Consensus amongst session participants:

- Box model of Gobas & Arnot is sufficient
- Gobas model's parameter 'Kmet' or whole-fish biotransformation rate of the parent molecule, provides the key metric that modifies Kow-only based 'B' estimates
- Metabolic rates from sub-cellular, cellular and in situ-preps can be scaled to estimate Kmet, hence also assist the prediction of 'B'

Breakout Session II: Application of *in vitro* data

Other Data

Several studies have been performed using whole fish, where uptake, elimination and metabolism rates have been determined (e.g., Dick Sijm)

→ rates from these studies provide an important set of data that can greatly assist the analysis of *in vitro* to *in vivo* extrapolation

Breakout Session II: Application of *in vitro* data

General ideas / questions raised etc.

- Demonstrating usefulness of (*in vitro*) methods should be based on the same species used to assess 'B'
- Standardisation of protocols necessary
- Chemical analysis: Measurement of free and sorbed fractions

  (e.g. also in S9 and microsomal media)

Overall prospectus is based on the Gobas and Arnot fish bioaccumulation model: Kmet (rate of whole-body metabolism) = key parameter that can alter BCF/BAF based on Kow-only calculations

Session participants

- Scott Dyer, Procter & Gamble
- John Nichols, USEPA
- Kevin Kleinow, Louisiana State Univ.
- Margaret James, Univ. Florida
- Kanaan Krishnan, Univ. Montreal
- Jean Domoradzki, Dow Corning
- Paul Jean, Dow Corning
- Jasminder Sahi, CellzDirect
- Margo Moore, Simon Fraser Univ.
- Luba Vasiluk, Simon Fraser Univ.
- Roman Lanno, Ohio State Univ.
- Birgit Hoeger, ECVAM

Breakout session II: Decision Tree

***Session Charge*:**

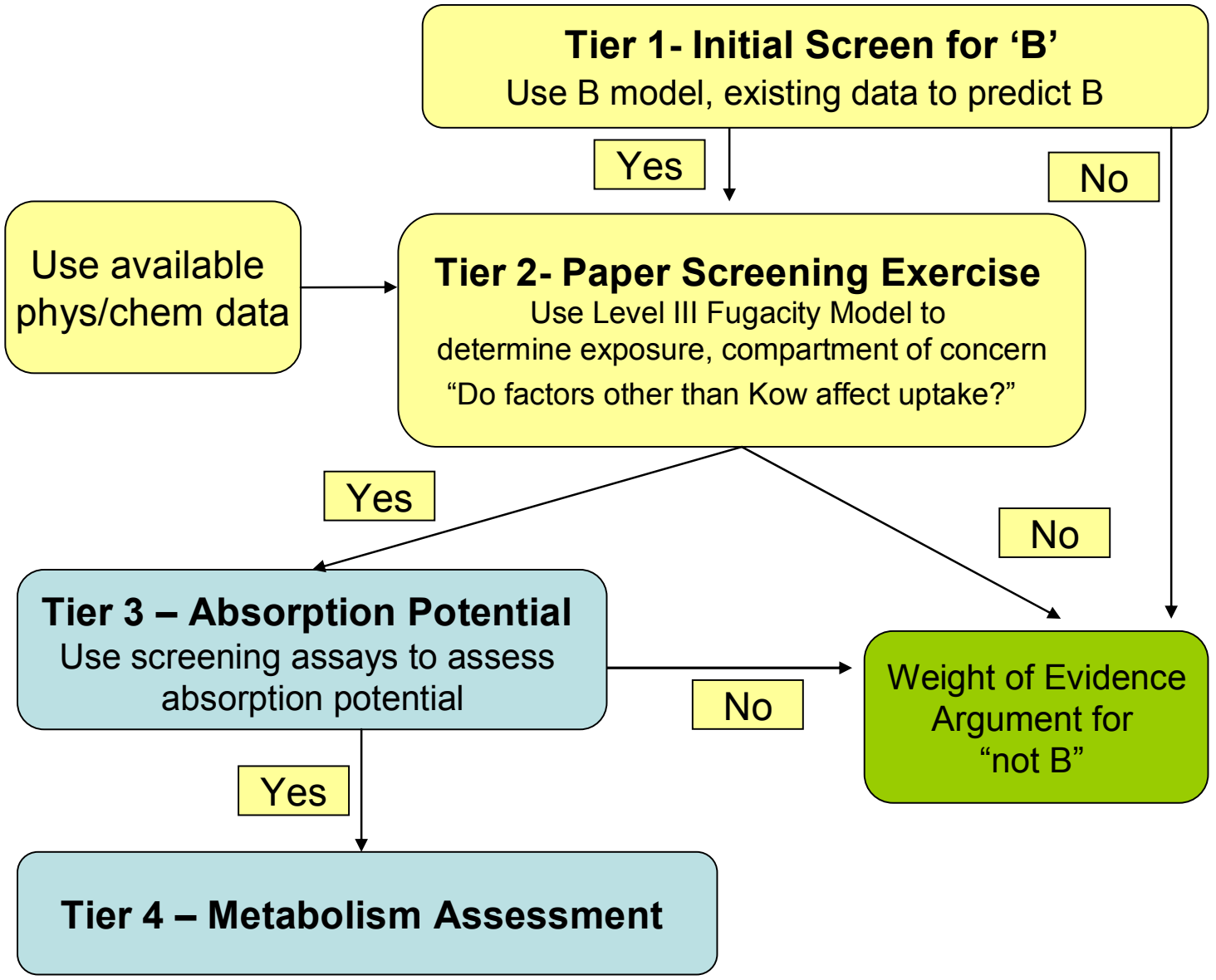
design a decision tree that can be used to assess the potential for bioaccumulation in fish

- To maximize our understanding of bioaccumulation potential while minimizing the use of animal testing.
- Minimize testing requirements through the use of existing physicochemical data.
- Reduce the time and resources needed for chemicals to be evaluated.
- Improve prioritization and identification of chemicals for further study.
- Increase understanding of structure activity relationships (SAR).

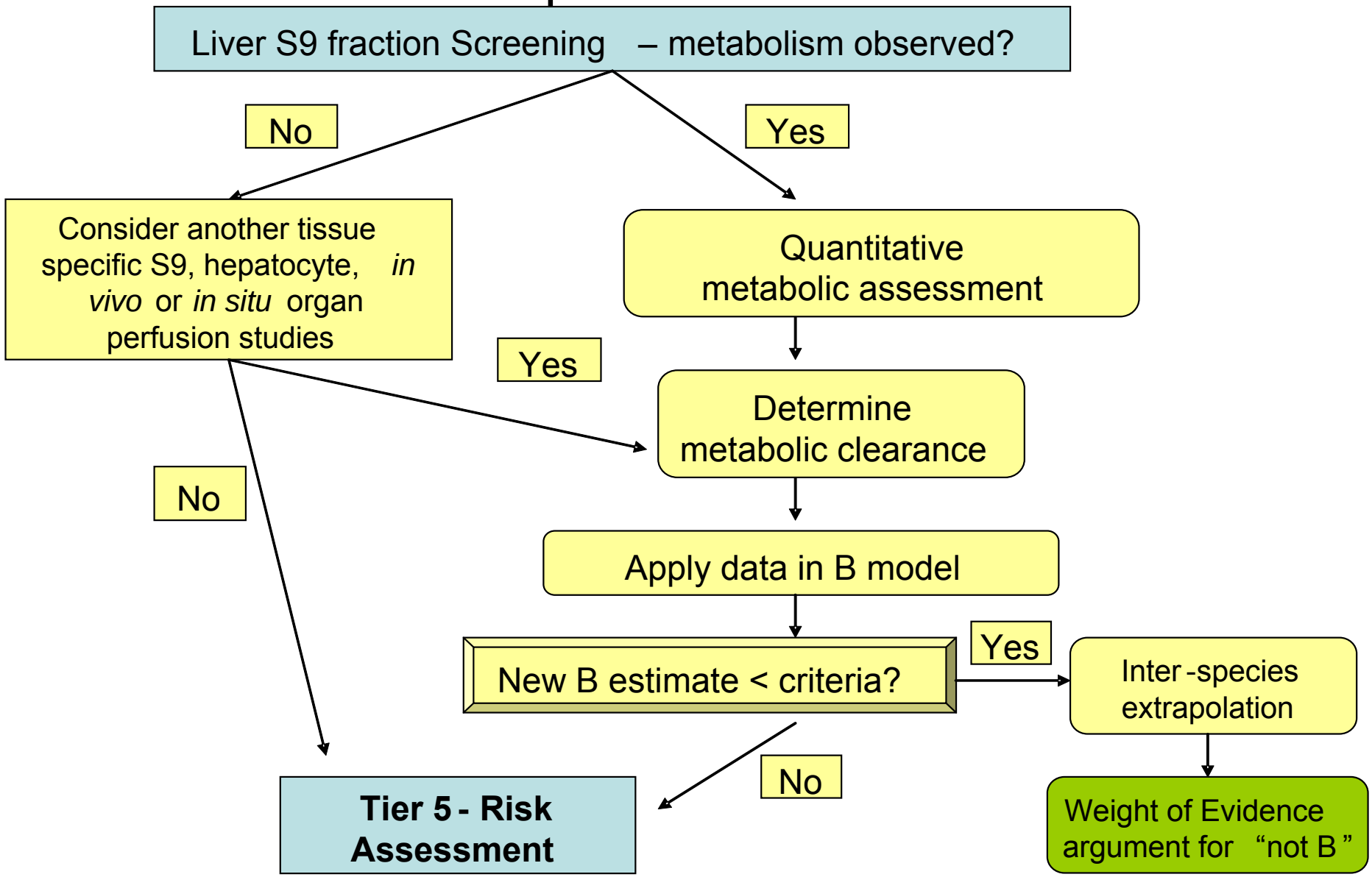
***Expected outcome*:**

A "Decision Tree" which provides a step-by-step guide to decisions on which next steps to take when determining the potential for "B"

Overall "B" Decision Tree



Metabolism Assessment Decision Tree Expanded Tier - 4



Participants

- Chris Cowan, Proctor and Gamble
- Curtis Eickhoff, Vizon Inc.
- Duane Hugget, Pfizer
- Helmut Segner, University of Bern, Switerland
- Irv Schultz, Battelle Laboratories
- Kurt Werner, 3M Corporation
- Peter Landrum, NOAA
- Kathy Plotzke, Dow Corning – Leader

Final Comments

- Research Strategy and Decision Tree Built on Consensus
  - Dedicated, focused group of participants
- Proceedings will be published as a short paper in Environmental Health Perspective – within 6 months of the workshop
  - Quickly get the word out
  - Longer, more detailed publications to follow as needed
- Copies of outcomes available as part of your thought starters

Thank you for your attention!